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Library-Based Development of New Optical Imaging Probes

Young-Tae Chang's group has expanded their library of novel rosamine fluorophore analogs synthesized by combinatorial solid-phase chemistry. These probes display a wide range of quantum efficiencies, and absorbance and emission wavelengths. The rosamine library has been screened in cell imaging and bio-analyte response assays (Wang *et al*., *JACS*, 2006). A major effort has been directed towards screening this library against a bead-attached peptide library, in order to identify specific and high affinity fluorophore-peptide pairs, to be eventually used for site-specific protein labeling in living cells. A 10^6-member library of 10-mer peptides was screened against the rosamine library, and the brightest peptide beads were selected on a fluorescence scanner with UV irradiation. These peptides were sequenced, re-synthesized on a large scale, and tested again for binding to the rosamine compound. One peptide-rosamine pair has been found with 4 μM dissociation constant and a 15-fold increase in fluorescence intensity upon binding. We are now optimizing the peptide sequence and length and completing our characterization of this labeling system.

Paul Clemons' group has performed high-throughput optical characterization of the Chang fluorophore libraries using a Varioskan fluorescence monochromator. Compounds were compared based on the locations of excitation and emission peaks, maximum fluorescence intensities, and integrated volumes under the two-dimensional surface of fluorescence intensities. Production conditions for RNA construct preparation were developed for aptamers recognizing known small molecules, as well as for derivative RNA diversity libraries. Shifts of emission wavelength and increases in fluorescence intensity were observed for several compounds among the Chang group rosamine library upon RNA binding. Screening experiments addressed reproducibility by performance in triplicate, RNA concentration-dependence by using multiple RNA aptamer concentrations, and specificity by counterscreening with both a random RNA species and yeast total RNA. Conditions were also established for surface plasmon resonance (SPR) characterization of RNA-small molecule binding events to provide evidence of physical binding independent of fluorescence intensity. In order to choose the best compounds for cell-based labeling, high-content screening experiments to assess the fluorescence properties and persistence of signal in live cells are underway. Using compounds with appropriate cellular properties, future work will involve SELEX starting from RNA diversity libraries based on the malachite green aptamer stem-loop structure, with improvements in binding measured by SPR, and improvements in fluorescence properties measured by fluorescence monochromator.

Alice Ting's group has improved biotin ligase-based targeting of quantum dots (QDs) to specific cellular proteins (Howarth *et al*. *PNAS* 2005 and Howarth *et al*., *Nature Methods*, 2006) as follows. (1) Improved QD reagents which are smaller and monovalent have been developed and used for labeling of cell surface EGF and LDL receptors. (2) Using phage display evolution, a new peptide substrate has been found for yeast biotin ligase. This new enzyme-substrate pair is orthogonal to the *E. coli* biotin ligase-acceptor peptide pair, allowing labeling of two different

protein targets expressed on the same cell with two different probes.

In addition, the Ting group reports another labeling method based on the enzyme transglutaminase (Lin *et al.*, *JACS*, 2006).

Publications

Howarth, M., Takao, K., Hayashi, Y., Ting, A.Y. 2005. Targeting quantum dots to surface proteins in living cells with biotin ligase. *Proc. Natl. Acad. Sci. USA* **102**: 7583-7588.

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Lin, C.-W., Ting, A.Y. 2006. Transglutaminase-catalyzed site-specific conjugation of small-molecule probes to proteins *in vitro* and on the surface of living cells. *J. Am. Chem. Soc.* **128**: 4542-4543.

Wang, S.; Chang, Y.T. 2006. Combinatorial synthesis of benzimidazolium dyes and its diversity directed application toward GTP-selective fluorescent chemosensors. *J. Am. Chem. Soc.* **128**: 10380-10381.